Brine shrimp lethality assay with selected medicinal plants extracts

S. Suneka[1], T. Manoranjan*[1]

[1]Department of chemistry, Faculty of science, University of Jaffna

**Email:* tmranjan@univ.jfn.ac.lk

Abstract— Brine Shrimp Lethality Assay (BSLA) is a preliminary and important screening tool for cytotoxicity. This assay monitors toxic substances in plant extract and other based solvents. The toxic substance kills laboratory cultured larvae (nauplii). *Artemia salina* (nauplii), the first larval stage of many crustaceans, having an unsegmented body and a single eye. It is about 22 mm long. Organic extracts of the three terrestrial medicinal plants were investigated for the biological activity against BSLA with the help of *Artemia salina*. Those selected plant extracts were also tested for phytochemical analysis. The goal of the study is to evaluate the cytotoxicity of each plant extract and compare the activity among them by using BSLA test data. For the analysis, dried plant leaves and aerial parts were grounded into fine powder. About 500 grams of powder was extracted by cyclohexane, dichloromethane (DCM) and methanol respectively. The extract were filtered and concentrated in rotatory evaporator and each crude weight were recorded. According to the BSLA test of these plant extracts, the DCM extracts of *Tephrosia purpurea* (Sarphonka (*E*), Kavila (*T*)), *Andrographis paniculata* (Kings of bitter (*E*), Nilavembu (*T*)) and *Oldenlandia umbellata* (Chay root (*E*), Chaaya ver (*T*)) showed high activity than other solvents extracts. The LC_{50} (fifty percentage lethality concentration) value of dichloromethane extracts of *T. purpurea* was shown high value as 104.712 ppm, while *A. paniculata* and *O. umbellate* were shown 125.89 ppm and 223.872 ppm respectively. This result indicates the variety of active compound/s responsible for activity in the extracts especially in the DCM extracts.

Keywords— BSLA, *Artemia salina, Tephrosia purpurea, Andrographis paniculata , Oldenlandia umbellata*

I. INTRODUCTION

Traditional medicine has an important role in disease prevention, defusing symptoms and cure. Plants are used for the treatment of many ailments and around 85% of the traditional herbal medicines used for primary healthcare treatments (Gafna, 2014, Qadir *et al*, 2015).

Traditional medicine has been practiced in Sri Lanka for more than 3,000 years. Eventually, recorded information about the herbals helps to improve the knowledge in the usage of specific medicinal plants and the methods of application. Majority of people in Sri Lanka use traditional medicines for their health care needs (Jooste, 2012).

BSLA can be used to check the toxicity of various active compounds in plants (Sarah *et al.,* 2017). This method is very simple, inexpensive and attractive method. Currently, *Artemia salina* (nauplii) is used as a model organism in various toxicity tests for BSLA and it is the major taxon in many hyper saline biotypes throughout the world feeding primarily on phytoplankton (Libralato *et al.,* 2016). Therefore, BSLA has been used to screen out the toxicity level of those crudes (Lilybeth *et al*, 2013). The present investigation was carried out to determine the toxicity against *A. salina* by using different crude extracts of *A. paniculata*, *T. purpurea* and *O. umbellate,* to determine the 50% lethality concentration. The phytochemical analysis was also carried out to the plant's extracts and finally to separate the active components from the extracted crude with more cytotoxicity against *Artemia salina*.

II. MATERIALS AND METHODOLOGY

A. Sample collection

Fresh leaves of *T. purpurea*, *A. paniculata* and aerial parts of *O. umbellata* were collected from a land area in Kurukkalmadam, Batticaloa, Sri Lanka. The taxonomic identities of these plants were confirmed by using herbarium samples, which were deposited in our department and the images on the internet. These plant leaves and aerial parts were washed well to remove foreign particles and those air dried for two weeks. The air dried parts were finely powdered separately and kept in closed plastic containers at room temperature.

B. Preparation of plant extracts

500 grams of powder sample was weighed out from each plant and placed into 2.5 L black reagent bottles and 1.25 L of cyclohexane was added. Then the container was occasionally shacked for period of 24 hours in room temperature and filtered. The solvent was removed by using rotatory evaporator and weighed. Finally, extracted crude was collected into a Macconky bottle. Then the plant residues were sequentially extracted by using dichloromethane and followed by methanol and their crude weight were recorded.

C. Brine Shrimp Lethality Assay (BSLA)

C.I. Hatching of Brine shrimp

Sea water was collected from Mathahal coast, Jaffna, Sri Lanka, and it was filtered and sterilized for 20 minutes in an autoclave and allowed to cool to room temperature. Then the sea water was strongly aerated for 24 hours. This sea water

was taken into the Petri dish and a few grams of *Artemia salina* cyst were added. Then it was allowed to hatch for 48 hours under a light source.

C.II. Solution preparation for BSLA

150 mg of crude (dried plant powder) was dissolved in 750 µL of cyclohexane and obtained 2×10^5 ppm solution. It was serially diluted with sea water and different concentrations of solutions (200 ppm, 100 ppm, 50 ppm, 25 ppm, 12.5 ppm) were prepared. For each extract, 20 mL of control solution was prepared with 750 µL of cyclohexane. For each plant extract, duplicate solutions were prepared.

C.III. Cytotoxicity test

D. Identification of Macroinvertebrates

The prepared solutions were transferred into different Petri dishes. 10 numbers of brine shrimp nauplii (*Artemia salina* nauplii) were transferred into each solution by using a dropper. All petri dishes were kept under light for 24 hours at room temperature. After 24 hours' period, live nauplii were counted to find out lethality percentage using the following equation:

$$Leathality\ \%\ of\ brine\ shrimp = \frac{Number\ of\ dead\ nauplii}{Total\ number\ of\ nauplii}\ x\ 100$$

Phytochemical analysis was carried out by using standard procedure (Sithara *et al*., 2016 and Gul *et al.,* 2017). Flash column chromatography was done for DCM extract with cyclohexane and DCM solvent system.

III. RESULTS AND DISCUSSION

The present study was performed to find out toxicity level and the presence of phytochemicals considered as active medicinal chemical constituents. Most common bioactive compounds are alkaloids, saponins, flavonoids, tannins, glycosides, steroids and terpenoids. The following table 1 indicates the results of our phytochemical analysis.

A. Phytochemical analysis

According to the phytochemical analysis most active compounds were found to be DCM and methanol extracts. Normally secondary metabolites compounds are responsible for bio activity. In *A. paniculata*, diterpenoid lactones are the commonest terpenoid compounds isolated from the aerial parts and roots of this plant (Okhauarobo *et al*., 2014). The previous phytochemical analysis of petroleum ether extract of *A. paniculata* indicates that the presence of phenolic compounds, flavanoids, alkaloids and saponins while absent in steroids and tannins. In the case of chloroform extract phenolic compounds, flavanoids, alkaloids, steroids, saponins and tannins were found (Sithara *et al*., 2016). Our current study proved that phenolic compounds, tannins, flavonoids, saponin, steroids and alkaloids are present in the DCM extracts of this plant.

B. Cytotoxicity assay result

Table 1: Phytochemical analysis results of plant extracts; + active

Test	*Andrographis paniculata*			*Tephrosia purpurea*			*Oldenlandia umbellata*		
	C	D	M	C	D	M	C	D	M
Sugar	-	+	+	-	+	+	-	+	-
Starch	-	-	+	-	-	+	-	-	+
Phenol/tannins	-	+	+	-	+	+	-	+	+
Flavonoids	-	+	-	-	+	+	-	+	-
Saponin	-	+	+	-	+	+	-	+	-
Glycosides	+	-	-	+	+	-	-	-	-
Steroids	-	+	-	-	-	-	-	+	-
Terpenoids	+	-	-	+	-	-	+	-	-
Alkaloids	-	+	+	-	-	+	-	-	+

C: Cyclohexane, D: Dichloromethane/DCM, M: Methanol)

The lethality assay was conducted on three terrestrial medicinal plants. Crude extracts were obtained using cyclohexane, dichloromethane and methanol. The Lethal concentration (LC_{50}) values were determined using Origin pro (version 8.5). The percentage mortality in the form of profit value vs. $\log_{10}$ of the various concentrations was plotted.

C. Andrographis paniculata

Table 2: Cytotoxicity assay result for extracts of *A. paniculata*; C: cyclohexane, D: DCM, M: Methanol and - inactive

Concentration (ppm)	Total number of Nauplii	Number of mortality of Nauplii			Lethality percentage (%)		
		C	D	M	C	D	M
0 (control)	20	0	0	0	0%	0%	0%
12.5	20	0	1	0	0%	5%	0%
25	20	0	3	1	0%	15 %	5%
50	20	3	4	3	15 %	20 %	15 %
100	20	5	6	6	25 %	30 %	30 %
200	20	8	15	7	40 %	75 %	35 %

From the result, the LC_{50} value of cyclohexane, DCM and methanol were 158.893 ppm, 125.893 ppm and 141.254 ppm respectively.

D. Tephrosia purpurea

From the result, the LC_{50} value of Cyclohexane, DCM and Methanol were 281.838 ppm, 104.712 ppm and 199.526 ppm respectively.

Table 3: Cytotoxicity assay result for extracts of *P. purpurea*; (C: Cyclohexane, D: Dichloromethane, M: Methanol)

Concentration (ppm)	Total number of Nauplii	Number of mortality of Nauplii			Lethality percentage (%)		
		C	D	M	C	D	M
0 (control)	20	0	0	0	0%	0%	0%
12.5	20	0	0	1	0%	0%	5%
25	20	0	4	2	0%	20%	10%
50	20	0	5	4	0%	25%	20%
100	20	0	7	7	0%	35%	35%
200	20	0	16	10	0%	80%	50%

E. Oldenlandia umbellata

Table 4: Cytotoxicity assay result for extracts of *O. umbellata*; (C: Cyclohexane, D: DCM, M: Methanol)

Concentration (ppm)	Total number of Nauplii	Number of mortality of Nauplii			Lethality percentage (%)		
		C	D	M	C	D	M
0 (control)	20	0	0	0	0%	0%	0%
12.5	20	0	0	0	0%	0%	0%
25	20	0	0	0	0%	0%	0%
50	20	0	2	0	0%	10%	0%
100	20	0	4	0	0%	20%	0%
200	20	0	4	0	0%	20%	0%

From the result, the LC_{50} value of DCM was 223.872 ppm while Cyclohexane and Methanol were not express significant results.

According to the results in the tested plant extracts, DCM extract has shown higher response for the BSLA than cyclohexane and methanol extracts. DCM extracts of *A. paniculata*, *T. purpurea* and *O. umbellata* have shown significant response to the cytotoxicity assay with brine shrimp. *A. paniculata* and *T. purpurea* showed higher activity than *O. umbellata* in all extracts.

Generally, LC_{50} value ranges of 100 -500 µg/ml are medium toxic and the values within the range of 0 -100 µg/ml are highly toxic. Therefore, we can identify the toxic component containing extracts and determine the level of toxicity against the *Artemia salina*.

DCM extract of *T. purpurea* has shown 80% of mortality with the corresponding LC_{50} value of 104.712 ppm. Therefore, it was near to high toxic range. At the same time DCM extract of *A. paniculata* showed that the LC_{50} value of 125.893 ppm. This was coming under the mild toxic range.

The methanol extracts also showed significant results for this assay. The methanol extract *A. paniculata* exhibited LC_{50} value 125.893 ppm while *T. purpurea* showed value LC_{50} value 199.526 ppm respectively. According to the result, *A. paniculata* has shown higher cytotoxic activity than *T. purpurea*.

The plant extracts of *O. umbellata* did not show activity than the other two plant extracts. Although DCM extract of *O. umbellata* showed the LC_{50} value as 223.872 ppm, it comes under the mild toxic range. .

IV. CONCLUSION

The present study showed that the DCM extracts of selected three plants have active compounds with cytotoxicity against *Artemia salina.* The high cytotoxic activity was being shown by the DCM extracts of *T. purpurea* while *A. paniculat* has shown mild cytotoxic effect. The mild toxic effects were being shown by methanol and cyclohexane extracts of *A. paniculata* and *T. purpurea*. While *O. umbellate* showed less cytotoxic effect.

The methanol extracts cytotoxic activity results in decreasing order is *A. paniculata*, *T. purpurea* and *O. umbellata*.

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